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Richard J. Lee      7/31/88  
PI - Signature      Date

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## INTRODUCTION:

Among women living in western societies, breast cancer is the most common malignancy with one of the highest mortality rates. Retinoids currently offer a promising alternative modality to antiestrogens. The field of retinoids, analogues of Vitamin A, has been expanding, and these compounds contribute to the growth and differentiation of many normal tissues. Retinoids and antiestrogens are small hydrophobic ligands, and interact with members of the steroid/thyroid receptor superfamily. These nuclear receptors (e.g., estrogen: Estrogen Receptor [ER]; retinoids: Retinoic Acid Receptor [RAR] & Retinoid X Receptor [RXR]) are transcription factors that are activated allosterically by ligand binding turning on their respective transcriptional function. The ligand-bound receptor then forms a hetero- or homo- dimer complex to initiate the transcription of genes that may, in part, mediate the inhibition of breast cancer proliferation. As in any form of chemoprevention, acquisition of resistance to retinoids can be a problem. It is plausible that an alteration in the common pathways shared by both retinoids and antiestrogens may develop into simultaneous resistance to both classes of drugs. Coregulators are proteins or RNA that are involved in many of the nuclear receptor pathways. They facilitate the promotion or repression of the transcription of hormone driven genes through the deacetylation and acetylation of histones. The exact mechanism still remains unclear. We propose to identify the coregulators and retinoid related genes that could contribute to the acquisition of retinoid resistance in breast cancer. We will use gene microarray to study the expression of these co-regulators and to identify pathways and biomarkers of acquired retinoid resistance and cross-resistance in breast cancer, and we will use the yeast two-hybrid system to find novel coregulators.

## PURPOSE:

*We hypothesize* that the loss/gain of coregulator(s) that interact with RAR $\alpha$  is responsible for the acquisition of resistance to RAR/RXR selective retinoids in breast cancer.

## TECHNICAL OBJECTIVES:

**Specific Aim 1.** We will select estrogen independent MCF-7/LCC1 cells against retinoids, 4HPR [unclear specificity], 9-cis-RA [pan RAR and RXR agonist], and TTNPB [RAR selective agonist]. We will evaluate the resistance and sensitivity of the newly derived and established cell lines to RAR/RXR selective retinoids and antiestrogens, TAM [ER partial agonist] and ICI 182,780 [ER antagonist], and will measure retinoid and estrogen receptor levels. We will use the cell lines to further investigate the pattern of expression of the genes identified in Aim 2.

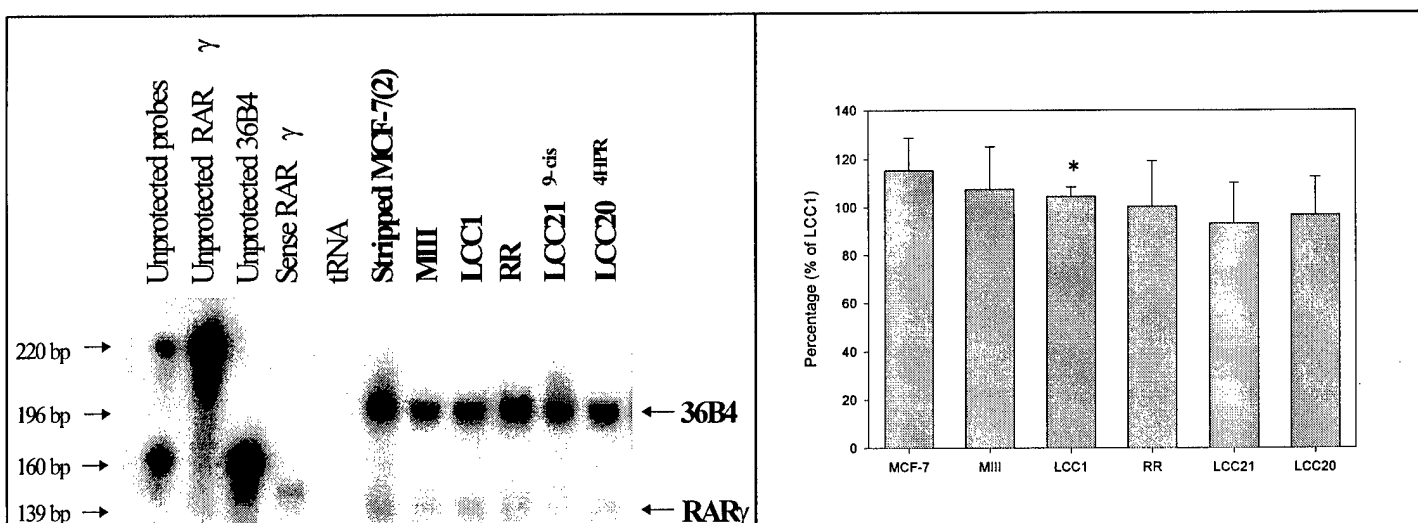
**Specific Aim 2.** We will identify known coregulator(s) that interact(s) with the RAR $\alpha$  (Retinoic Acid Receptor) using gene microarray. We will verify and examine their patterns of expression in the retinoid sensitive cell lines MCF-7 and MDA435/LCC6 and in the established retinoid resistant cell lines, MDA-MB-231, BT-20, and MCF-7/RR [generated by selection against tamoxifen], and in the newly generated retinoid resistant ER+ cell lines from Aim 1.

## Summary

In the second year, Technical Objective 1 is the main focus of the project and is close to being completed. Retinoid generated resistant cells are necessary to study acquired retinoid resistant pathways. Stock cultures of mammary epithelial cancer cells, MCF-7, were maintained in BioFluids IMEM (Improved Minimal Essential Media) with phenol red supplemented with 5% fetal bovine serum in a humidified atmosphere containing 5% CO<sub>2</sub> and 95% air at 37 °C. RR, MIII, LCC1, LCC20<sup>HPR</sup>, and LCC21<sup>9CIS</sup> were maintained in BioFluids IMEM without phenol red supplemented with 5% Charcoal Stripped Calf Serum.

Estrogen independent MCF-7/LCC1 was selected stepwise against 4HPR [unknown receptor specificity] and 9-cis-RA [ $K_d$  (nM) RAR $\alpha$ =11,  $\beta$ =7,  $\gamma$ =22; RXR $\alpha$ =9,  $\beta$ =11,  $\gamma$ =16]. 4HPR is presently used in phase II clinical trials because of its low toxicity and effectiveness. *Veronesi et al.* (JNCI 91: No. 21, 1847-1856, 1999) reported that 4-HPR is effective in reducing local recurrence and contralateral breast cancer in premenopausal women with early breast cancer. 9-cis-RA is a potent pan agonist for the RAR and RXR. These two drugs, 4-HPR and 9-cis-RA, were ideal choices to create cell line models of acquired retinoid resistance for human breast cancer due to their potential use in the clinic. The two generated cell lines were named LCC20<sup>HPR</sup> and LCC21<sup>9CIS</sup>. LCC20<sup>HPR</sup> was selected stepwise against 4HPR up to a concentration of 15  $\mu$ M, and then the drug was removed from the cells. LCC21<sup>9CIS</sup> was selected stepwise against 9-cis-RA up to a concentration of 10  $\mu$ M and 9-cis-RA was removed from the cells. After removal of the drug from the two different variants, the cells were allowed to grow without the presence of the drug for 30-60 passages. LCC21<sup>9CIS</sup> was confirmed to be resistant to 9-cis-RA, but showed no sign of cross-resistance to 4-HPR with respect to its parental cell line MCF-7/LCC1. However, LCC20<sup>HPR</sup> is resistant to 4-HPR and interestingly, it is cross-resistant to 9-cis-RA with respect to its parental cell line LCC1. The cross-resistance data suggests that the pathway(s) used in acquiring resistance to 4HPR may overlap the pathway(s) used in acquiring resistance to 9-cis-RA. Currently, the LCC1 cell line is being selected against other clinically useful retinoids TTNPB [ $K_d$  (nM) RAR $\alpha$ =20,  $\beta$ =39,  $\gamma$ =51; RXR $\alpha$ =8113,  $\beta$ =4093,  $\gamma$ =2566] and LG1069 [ $K_d$  (nM) RAR $\alpha$ >5000,  $\beta$ >5000,  $\gamma$ >5000; RXR $\alpha$ =27,  $\beta$ =44,  $\gamma$ =44]. Under treatment of TTNPB, cells' morphology is altered. They appear enlarged and flattened as the cells adapt to their treated environment.

Measurements of retinoid receptor expression profiles on LCC20<sup>4HPR</sup> and LCC21<sup>9CIS</sup> are currently in progress. In the first year, we measured the RAR $\alpha$  and RXR $\alpha$  expression in the retinoid resistant cell lines. We found that there was no change in RAR $\alpha$  expression among retinoid resistant cells, and there was a significant reduction in RXR $\alpha$  when LCC1 cells acquire 9-cis-RA resistance. Receptor profiles were measured with RNase Protection Assays using radiolabeled RNA probes. RNase Protection Assays of the retinoid resistant cell lines with RAR $\gamma$  riboprobes have thus been completed. At least three independent experiments were performed. For the RPA, all cells were fed and stripped with 5% CCS IMEM. Stripping MCF-7 was done as follows; for three days, MCF-7 was washed with 5% CCS IMEM three times on the first day of stripping and washed stepwise down for the following two days. On the fourth day, RNA was extracted from the cells. RNA was obtained using Trizol Reagent (Life Technologies, Inc., Grand Island, NY) according to manufacturer's instructions. The RAR $\gamma$  probe was kindly provided by Dr. Marco Gottardis and Dr. William Lamph from Ligand Pharmaceuticals. The RAR $\gamma$  probe was made by subcloning 140 bp of the RAR $\gamma$  cDNA into Promega's PGEM vector, and the 36B4 loading control was obtained similarly from 220 bp of the 36B4 cDNA. Riboprobes were labeled with [ $\alpha$ -<sup>32</sup>P]UTP. RPAs were performed as follows. Briefly, RNA (30  $\mu$ g), 36B4 probe, and retinoid probes were hybridized overnight at 50 °C followed by digestion with RNase A. Protected fragments were separated by gel electrophoresis on a 6% acrylamide Tris-Borate-EDTA/UREA gel (NOVEX, San Diego, CA). The gel was then vacuum dried at 80 °C for 1 hr. The data was quantified by phosphorimaging screens and visualized with autoradiography.



**Figure 1: A)** RNase Protection Assay of MCF-7 and its retinoid resistant variants probing with the RAR $\gamma$  riboprobe. **B)** Densitometric analysis of RAR $\gamma$  in the acquired retinoid resistant cell lines and their parental cell lines. The data are the means  $\pm$  SE of at least three independent experiments. (\*) Parental cell line for LCC20<sup>4HPR</sup> and LCC21<sup>9-cis</sup>.

The RAR $\gamma$  levels appear unaltered in the retinoid resistant cell lines, LCC20<sup>4HPR</sup> and LCC21<sup>9-cis</sup> (n=3). Densitometric analyses confirm that there are no significant changes in RAR $\gamma$  expression among the parental and resistant cell lines. The loss of retinoid receptor RAR $\gamma$  expression does not appear to be responsible for the acquired resistance to retinoids. Further, analysis of the expression levels of other retinoid receptors is currently underway.

To further characterize the cells, we are currently using cDNA microarrays on LCC20<sup>4HPR</sup> and LCC21<sup>9-cis</sup>. We are using Clontech's Human Atlas Arrays that contain human cancer related genes to explore differential expression of genes between the parental cell line LCC1 and its retinoid resistant derivatives. So far, we have done multiple repetitions on each of the cell lines using Clontech's Atlas Array. Each probe was generated from an independent cell culture, each culture being grown on different days using identical culture conditions. mRNA was isolated from proliferating subconfluent monolayers of each cell line. mRNA quality was determined by standard spectroscopic and gel electrophoresis analysis. Probes for the Clontech Atlas gene microarrays (Clontech, Palo Alto, CA) were prepared as described by the manufacturer. Clontech's Atlas Arrays were then prehybridized, hybridized with labeled cDNA, and washed as described by the manufacturer. Atlas Array was sealed in plastic and signals detected by phosphorimage analysis.

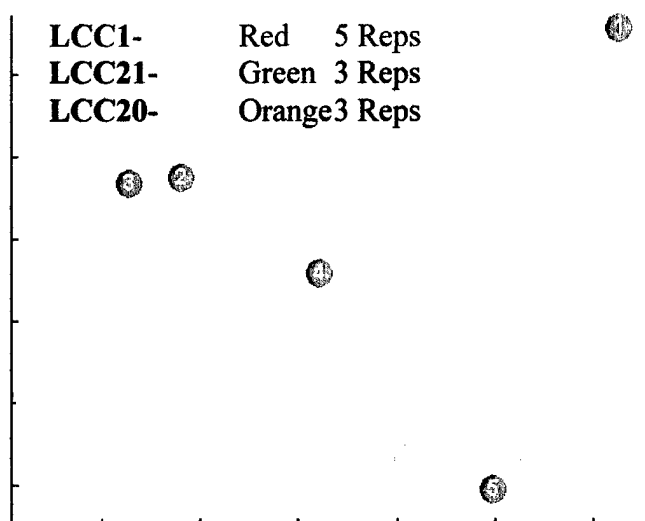
Background signal was estimated by selecting a random, preselected area of the filter, where there is no target cDNA. The value was subtracted from all "specific" signals, producing the background-corrected estimates. The data were then normalized to account for differences in probe specific activity, hybridization, and other variables among replicates. The data from these arrays should help to identify genes that may contribute to acquired resistance to retinoids in breast cancer cells.

	LCC1	LCC20 <sup>HPR</sup>	LCC21 <sup>9CIS</sup>
<b>prohibitin</b>	1	0.30	0.29
<b>integrin beta 6</b>	1	0.093	0.33
<b>JAK1 (protein-tyrosine kinase )</b>	1	0.30	0.25
<b>TNF receptor 1</b>	1	0.25	0.25
<b>IRF 1</b>	1	0.21	0.16
<b>C-1</b>	1	*	5
<b>HM89</b>	1	*	4
<b>leuckocyte-associated molecule-1 alpha subunit (LFA-1 alpha)</b>	1	*	3
<b>ubiquitin</b>	1	*	3
<b>transcription initiation factor TFIID subunit (TAFII31)</b>	1	*	2
<b>helix-loop-helix protein Id-2</b>	1	3	*

**Table 1:** List of selected genes from Clontech's Human Atlas Array which are differentially expressed from the parental LCC1 cells [  $p < 0.1$ , LCC1 (n=5), LCC20<sup>HPR</sup> (n=3), LCC21<sup>9CIS</sup> (n=3). \* denotes that no statistical difference was detected. ]

Statistical analyses (t-tests) were done on each of the 597 genes on the Array comparing the parental cell line LCC1 and the retinoid resistant cell line. Genes of interest with  $p < 0.1$  were chosen and listed in Table I. Clustering analysis is a major topic in categorizing cell lines' molecular profiles. A visual representation of a multi-dimensional space containing 597 genes is difficult, but reducing the multi-dimensional space into a 2-D space simplifies and allows us to see the clustering of each cell lines' molecular profile (*Wang et al.*).

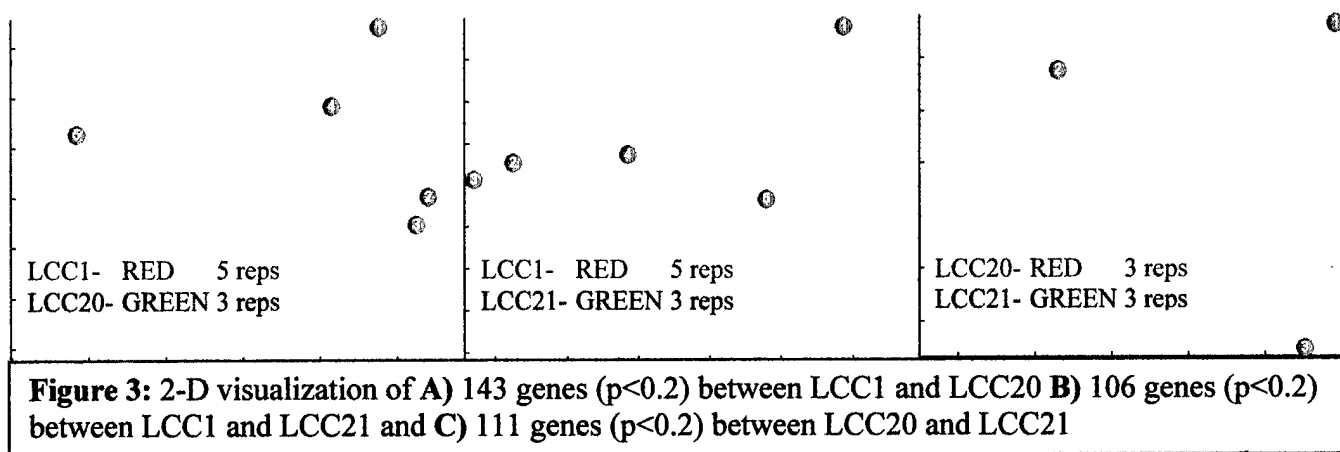
In Figure 2, based on all 597 genes on the Clontech's Atlas Array, the clustering of the three cell lines is non-linear. LCC1 clusters around the upper right hand corner; LCC20 occupies the lower left hand corner, whereas LCC21 clusters around the lower right hand corner of the 2-D visualization. Replicate number 5 from LCC1 may be an outlier from the other LCC1 replicates. More replicates are necessary to get a better representation to cluster the three different cell lines.



**Figure 2:** 2-D- Visual Representation of 597 genes from Clontech's Atlas Array. Each number represents the replicate that the experiment came from

Among the 597 genes, there are genes that are not detectable or show very little difference among the cell lines. These genes may obscure the 2-D visual representation. To reduce this obstruction, we can then choose specific genes based on a two-tailed t-test to see if the cell lines cluster better. Clustering analysis can also be done on specified genes. In Figures 3B) and 3C), one can draw a straight line to separate the two cell line clusters,





indicating linear separability. However, in Figure 3A), there is non-linear separability between the two cell lines since a line cannot be drawn to separate the LCC1 and LCC20 due to replicate #5 of LCC1. More replicates for each of the cell line is necessary to obtain a better 2-D visual representation of the sub-clustering analysis.

For Technical Objective 2, we have in our possession Origene's SmartArray. In this particular microarray chip, genes are specifically selected to be functionally related and are involved in the same regulatory pathway(s). We will use the Origene's SmartArray chip to address technical objective 2 because they have an exhaustive list of nuclear hormone receptors. These are known to differentially regulate gene expression depending upon which partner protein they bind with to form heterodimers. It also contains a list of transcriptional co-regulators (COR) which upon binding to the Nuclear Hormone Receptor (NHR) heterodimer and can function either to activate (co-activators) or inhibit (co-repressors) gene expression in response to ligand recognition. Third, the array contains an expanding group of secondary co-regulators (SCR) which, in concert with the NHR-co-regulator complex, act to either acetylate or deacetylate the chromatin and, ultimately, to either up-regulate or down-regulate expression of specific genes.

Searching for novel co-regulators using the yeast two-hybrid system has not been placed on the priority list due to technical difficulties. We obtained a considerable number of false positive clones using the RAR $\alpha$  bait; we have tried to reduce the number of false positives by using a different *Saccharomyces cerevisiae* strain from the Y190. The PJ strain has multiple reporter constructs that may limit the number of false positives; reproducibility was not consistent, so we took the alternative approach by examining the levels of known coregulators. To confirm results from the SmartArray, we obtained a fragment from each of the known coregulators (e.g., SRC-1, SMRT, and CBP) through PCR [Polymerase Chain Reaction]. We then inserted the PCR fragments into riboprobe vectors that will be used in RNase protection assays to study known coregulator level changes in the parental and acquired retinoid resistant cells. The assays will be performed once the retinoid receptor characterization is completed. We have not, however, terminated the yeast two-hybrid project because the technique offers the possibility of discovering novel coregulators.

We will continue to pursue the objectives in Aim 1. In addition, we will use the cDNA microarrays to characterize the newly generated cell lines. Meanwhile, we will examine the role of known and novel coregulators in these acquired retinoid resistant cell lines through RNase Protection Assays and the yeast two-hybrid system.

## APPENDIX

### *Bulleted List of Key Accomplishments*

- Established Acquired Retinoid Resistant Cell lines LCC20<sup>HPR</sup> and LCC21<sup>9CIS</sup>
- Anchorage Dependent Growth Assays of LCC20<sup>HPR</sup> and LCC21<sup>9CIS</sup> with 9-cis-RA and 4-HPR
- RNase Protection Assays of retinoid Resistant cell lines and parental with RAR $\alpha$ , RAR $\gamma$ , and RXR $\alpha$
- Constructed SMRT, CBP, and SRC-1 PCR fragments into riboprobe vectors.
- Microarray analysis on LCC1, LCC20<sup>HPR</sup> and LCC21<sup>9CIS</sup>

### *List of Reportable Outcomes*

#### Abstracts Presented at

- AACR Proceedings: The Steroid Receptor Superfamily: Celebrating the 10<sup>th</sup> Anniversary of the AACR Special Conference in Cancer Research: January 8-12, 1999. Renaissance Esmeralda Resort. Indian Wells, CA.
- AACR Proceedings:90<sup>th</sup> Annual Meeting: April 10-14, 1999. Volume 40. March 1999. Philadelphia, PA.
- Proceedings of the 1999 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. November 1999. Washington, DC
- AACR Proceedings:91<sup>st</sup> Annual Meeting: April 1-5, 2000. Volume 41. March 2000. San Francisco, CA.

## *Pubilcations*

Retinoid regulation of genes and growth in E2 independent breast cancer. Lee, R.Y., Baumann, K.H., Gottardis, M. M., Skaar, T.C., and Clarke, R. AACR Proceedings: 88<sup>th</sup> Annual Meeting: April 12-16, 1999. Volume 38: Abstract #3043, p. 455. March 1997. San Diego, CA.

Retinoid cross-resistance to 9-cis-RA and 4HPR is not associated with the loss of RAR $\alpha$  RNA expression. Richard Y. Lee, Todd C. Skaar, Fabio Leonessa, and Robert Clarke. AACR Proceedings: The Steroid Receptor Superfamily: Celebrating the 10<sup>th</sup> Anniversary of the AACR Special Conference in Cancer Research: January 8-12, 1999. Renaissance Esmeralda Resort. Indian Wells, CA.

The acquisition of retinoid resistance to 4HPR and 9-cis-RA in estrogen independent breast cancer. Lee, R.Y., Skaar, T.C., Leonessa, F., and Clarke, R. AACR Proceedings: 90<sup>th</sup> Annual Meeting: April 10-14, 1999. Volume 40: Abstract #406, p. 61. March 1999. Philadelphia, PA.

Retinoid cross-resistance to 9-cis-RA and 4HPR is not associated with the loss of RAR $\alpha$  and RXR $\alpha$  RNA expression. Lee, Richard Y., Skaar, Todd C., Gu, Zhiping, Leonessa, Fabio, and Clarke, Robert. Proceedings of the 1999 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. Published as a Supplement to Clinical Cancer Research, Volume 5: Abstract #242, November 1999. ISSN 1078-0432 Washington, DC.

Acquiring Resistance to 9-cis-RA and 4-HPR in Breast Cancer is not associated with RAR $\alpha$  and RXR $\alpha$  RNA expression. Lee, R.Y., Skaar, T.C., Gu, Z., Leonessa, F., and Clarke, R. AACR Proceedings: 91<sup>st</sup> Annual Meeting: April 1-5, 2000. Volume 41: Abstract #3924, p. 617. April 2000. San Francisco, CA.

The acquisition of retinoid resistance to 4HPR and 9-cis-RA in estrogen independent breast cancer. Lee, R.Y., Skaar, T.C., Leonessa, F., and Clarke, R. Georgetown University, Department of Physiology and Biophysics, Lombardi Cancer Center, 3970 Reservoir Rd., NW, Washington, DC 20007.

A possible mechanism of tumor progression that allows breast tumors to grow in postmenopausal women, who have low serum levels of estrogens, is through an acquisition of an estrogen independent phenotype. Chemopreventive drugs such as antiestrogens and retinoids are possible modalities to address this particular phenotype. Retinoids, analogs of Vitamin A, inhibit breast cancer cell proliferation and are useful chemopreventive agents for postmenopausal women. These compounds interact with receptors in the super-family of nuclear transcriptional factors. One issue with chemoprevention is the acquisition of resistance, but no established *in vitro* model has addressed the problem in acquiring retinoid resistance.

We have established two stable retinoid resistant cell lines (MCF-7/LCC20<sup>4HPR</sup> and MCF-7/LCC21<sup>9cis</sup>) by selecting an estrogen independent MCF-7 variant LCC1 against increasing concentrations of retinoids, N-(4-hydroxyphenyl) retinamide (4-HPR) and 9-cis retinoic acid (9-cis-RA). After growing more than 30 passages without retinoids, MCF-7/LCC20<sup>4HPR</sup> stably and consistently is 3-5 fold resistant to the drug 4-HPR, but shows a half log shift of cross-resistance to 9-cis-RA. However, MCF-7/LCC21<sup>9cis</sup> maintains its resistance to 9-cis-RA 100 fold, and also exhibits no cross-resistance to 4-HPR. Future studies are presently being directed towards looking at the molecular mechanisms of acquired retinoid resistance.

Retinoid cross-resistance to 9-cis-RA and 4HPR is not associated with the loss of RAR $\alpha$  RNA expression. Richard Y. Lee, Todd C. Skaar, Fabio Leonessa, and Robert Clarke. Georgetown University, Department of Physiology and Biophysics, Lombardi Cancer Center, 3970 Reservoir Rd., NW, Washington, DC 20007.

Retinoids, analogs of Vitamin A, inhibit breast cancer cell proliferation and are useful chemopreventive agents for postmenopausal women. These compounds have varied selectivity for retinoid receptors in the super-family of nuclear transcriptional factors. 9-cis retinoic acid (9-cis-RA) is a retinoid pan agonist that activates both RAR and RXR isoforms. N-(4-hydroxyphenyl) retinamide (4-HPR) has unclear receptor selectivity, but shows promising clinical activity and is presently used in phase I clinical trials. One issue with retinoid chemoprevention is the acquisition of resistance and possible cross-resistance, but no established *in vitro* model has been developed to study the problem of acquired retinoid resistance for postmenopausal breast cancer patients.

We established an *in vitro* model by generating two stable retinoid resistant cell lines, MCF-7/LCC20<sup>4HPR</sup> and MCF-7/LCC21<sup>9cis</sup>. They were generated through selection of an estrogen independent MCF-7 variant (LCC1) against increasing concentrations of 4-HPR and 9-cis-RA. Anchorage-dependent growth assays confirm that MCF-7/LCC20<sup>4HPR</sup> is stably and consistently 3-5 fold resistant to the drug 4-HPR, but shows a half log shift of cross-resistance to 9-cis-RA after growing more than 30 passages without the drug. However, MCF-7/LCC21<sup>9cis</sup> maintains its resistance to 9-cis-RA (100-fold), and also exhibits no cross-resistance to 4-HPR. To measure the RAR $\alpha$  RNA levels, we used a RNase Protection Assay with a riboprobe that is antisense to the nucleotide region 1798 to 1922 of the RAR $\alpha$  gene. RAR $\alpha$  RNA levels of these retinoid resistant cell lines are unaltered with respect to the parental cells. Future studies are presently being directed towards identifying the molecular mechanisms of acquired retinoid resistance and cross-resistance.

Retinoid cross-resistance to 9-cis-RA and 4HPR is not associated with the loss of RAR $\alpha$  and RXR $\alpha$  RNA expression.

Lee, Richard Y., Skaar, Todd C., Gu, Zhiping, Leonessa, Fabio, and Clarke, Robert. Georgetown Univ., Dept. of Physiology & Biophysics, Lombardi Cancer Center., Washington, DC 20007.

Retinoids, analogs of Vitamin A, inhibit breast cancer cell proliferation and are useful chemopreventive agents for postmenopausal women. These compounds have varied selectivity for retinoid receptors in the super-family of nuclear transcriptional factors. 9-cis retinoic acid (9-cis-RA) is a retinoid pan agonist that activates both RAR and RXR isoforms. N-(4-hydroxyphenyl) retinamide (4-HPR) has unclear receptor selectivity, but shows promising clinical activity and is presently used in phase II clinical trials. One issue with retinoid chemoprevention is the acquisition of resistance and possible cross-resistance, but no established *in vitro* model has been developed to study the problem of acquired retinoid resistance for postmenopausal breast cancer patients.

We established an *in vitro* model by generating two stable retinoid resistant cell lines, MCF-7/LCC20<sup>4HPR</sup> and MCF-7/LCC21<sup>9cis</sup>. They were generated through selection of an estrogen independent MCF-7 variant (LCC1) against increasing concentrations of 4-HPR and 9-cis-RA. Anchorage-dependent growth assays confirm that MCF-7/LCC20<sup>4HPR</sup> is stably and consistently 3-5 fold resistant to the drug 4-HPR, but surprisingly shows a half log cross-resistance to 9-cis-RA after growing more than 30 passages without the drug. However, MCF-7/LCC21<sup>9cis</sup> maintains its resistance to 9-cis-RA (100-fold), but exhibits no cross-resistance to 4-HPR. RNase Protection Assay was used to measure retinoid receptor RNA levels. RAR $\alpha$  and RXR $\alpha$  RNA levels of these retinoid resistant cell lines are unaltered with respect to the parental cells. A cDNA atlas array is being used presently to identify molecular pathways and biomarkers of acquired retinoid resistance and cross-resistance. The information from the arrays will find known genes that may play a role in acquired resistance to retinoids.

**ACQUIRING RESISTANCE TO 9-CIS-RA AND 4HPR IN BREAST CANCER IS NOT ASSOCIATED WITH THE LOSS OF RAR $\alpha$  AND RXR $\alpha$  RNA EXPRESSION.** R.Y. Lee, T. C. Skaar, Z. Gu, F. Leonessa, and R. Clarke. *Georgetown Univ., Dept. of Physiology & Biophysics, Lombardi Cancer Center., Washington, DC 20007.*

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